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Research Note—

The Effect of Killed Salmonella enteritidis Vaccine Prior to Induced Molting on the Shedding of S. enteritidis in Laying Hens

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SUMMARY. Effects of administering killed *Salmonella enterica* serovar *enteritidis* (SE) vaccines to laying hens prior to induced molting on egg production and on shedding of SE were investigated. Forty hens were vaccinated with one of two SE vaccines available commercially in the United States and Japan. Twenty-five days after vaccination, feed was withdrawn for 2 wk from 20 vaccinated plus 10 unvaccinated hens to induce molt. Four days after molt induction, all hens were challenged with a dose of 2.4×10^9 of SE. For the 25 days following administration of the SE bacterins, egg production in vaccinated hens showed approximately a 15% decrease. After molt induction, egg production in molted hens ceased and then returned to normal levels 8 or 9 wk postvaccination. Through the 3-mo experimental period, the decreases in numbers of eggs laid in the unvaccinated/molted group and two vaccinated/molted groups were 225 (26.2%), 245 (28.4%), and 274 (31.9%), respectively, compared with 860 in the unvaccinated/unmolted group. There was no significant difference in egg lay at the $P < 0.05$ level among the former three groups. Hens in the vaccinated/molted groups shed about two logs less SE than hens in the unvaccinated/molted group 3–14 days postchallenge ($P < 0.05$ or 0.01). These results indicate that vaccination prior to induced molting might be effective in preventing the exacerbation of SE problems within flocks in which the potential for SE contamination may exist.

RESUMEN. *Nota de Investigación*—Efecto de la vacuna inactivada de Salmonella enteritidis aplicada antes de la muda forzada sobre la eliminación de Salmonella enteritidis en ponedoras comerciales.

Se investigaron los efectos de la administración de vacunas inactivadas de *Salmonella enterica* serovariedad *enteritidis* en ponedoras comerciales antes de la muda forzada, sobre la producción de huevos y la eliminación de la *S. enteritidis*. Se vacunaron cuarenta ponedoras con una de dos vacunas de *S. enteritidis* disponibles comercialmente en los Estados Unidos y Japón. A los 25 días posteriores a la vacunación, se les retiró el alimento a 20 ponedoras vacunadas y a 10 ponedoras no vacunadas durante dos semanas para inducir la muda. A los 4 días posteriores a la inducción de la muda, se desafiaron la totalidad de las ponedoras con una dosis de 2.4×10^6 unidades formadoras de colonia de *S. enteritidis*. Durante los 25 días posteriores a la administración de las bacterinas de *S. enteritidis*, se observó una disminución aproximada del 15% en la producción de huevos en las ponedoras vacunadas. Después de la inducción de la muda, se observó un cese en la producción de huevos en las ponedoras en muda forzada, retornando a sus niveles normales a las 8 a 9 semanas posteriores a la vacunación. Durante el periodo experimental de 3 meses, la disminución en el número de huevos puestos en el grupo no vacunado sometido a la muda forzada y dos grupos vacunados sometidos a la muda forzada fue de 225 (26.2%), 245 (28.4%) y 274 (31.9%), respectivamente, comparado con 860 huevos en el grupo no vacunado y no inducido a la muda. No se observó una diferencia significativa ($P < 0.05$) en la producción de huevos en los tres primeros grupos. Se observó una disminución de dos logaritmos en la eliminación de *S. enteritidis* desde el día 3 al día 14 posterior al desafío ($P < 0.05$ o 0.01%) los grupos vacunados sometidos

a muda forzada al ser comparada con la eliminación observada en las ponedoras del grupo no vacunado sometido a muda forzada. Los resultados indican que la vacunación anterior a la inducción de la muda podría ser efectiva en las prevención de la exacerbación de los problemas de *S. enteritidis* en aquellos lotes potencialmente expuestos a contaminación por *S. enteritidis*.

Key words: bacterin, chicken, cecal dropping, oil adjuvant, *S. enteritidis*

Induced molting remains an important management tool for the layer industry as a means to maximize the effective laying life of a flock. Molting programs involve an estimated 75%–80% of the commercial flocks in the United States and Japan. Although there are many procedures to molt hens, the primary method for molt induction is to remove feed until hens drop a certain percentage of body weight (21). However, previous experimental studies showed that this procedure significantly depressed immunity in the hens (2,3), which may leave flocks vulnerable to infection by a number of infectious agents. One organism in particular, *Salmonella enterica* serovar *enteritidis* (SE), was studied extensively with regard to infection during molt. Molted hens shed more SE from their feces (4,5,8,10), had higher levels of SE in internal organs (8), remained infected for longer periods (4,9), exhibited more pathology in the intestinal tract than their unmolted counterparts (9,12,16), and caused the recrudescence of a previous SE infection (11).

The above results reflect the effects of induced molting on SE infection under experimental, controlled conditions and may not reflect what actually occurs in commercial field situations. However, several studies in the United States (20) and Japan (14) showed that the levels of environmental salmonellae increased dramatically in commercial flocks following a molt. These results provide some indication that molting may exacerbate an SE problem in commercial situations.

Vaccination has long been used as a management tool to protect poultry against a range of pathogens, including *Salmonella*. Recent studies showed the effectiveness of using a live attenuated *S. enterica* serovar *typhimurium* vaccine to reduce SE problems in hens undergoing molt (7), and the current study was undertaken to determine if administering hens an SE bacterin prior to molt will similarly provide a degree of protection to hens against SE challenge during a molt.

MATERIALS AND METHODS

Laying hens. Seventy Single Comb White leghorn hens at 70 wk of age were obtained from the commercial laying flocks of Aomori Poultry Co. Ltd. They were raised in individual layer cages in a totally light-controlled building exposed to a 17-hr light (L):7-hr dark (D) regimen and allowed *ad libitum* access to water and antibiotic-free feed (17% CP, 3.0% Ca, 0.55% P).

The hens were observed for 1 wk and 60 individuals were selected based on egg production and general health. The hens were divided into six groups: the control group (group 1); the group vaccinated with vaccine A (Layermune SE; Biomune, Lenexa, KS; group 2); the group vaccinated with vaccine B (Inactivac SE4; Maine Biological Lab, Waterville, ME; group 3); the molted group (group 4); the group vaccinated with vaccine A and molted (group 5); and the group vaccinated with vaccine B and molted (group 6). Groups 2, 3, 5, and 6 were administered vaccine subcutaneously as per label directions.

Molt procedure. Twenty-five days postvaccination chickens in groups 4, 5, and 6 were molted according to the procedure obtained from the supplier of the chickens (Aomori Poultry Co. Ltd.). Before the feed withdrawal, the chickens were exposed to 14.5 L:9.5 D. After the feed withdrawal, body weight was checked every day, and when the loss of body weight reached more than 30% (average loss of body weight of the chickens in groups 4, 5, and 6 was 32.9%, 30.8%, and 32.3%, respectively), the birds were returned to feed, using the same feed described above. On the initial day of refeeding and on the following day, each bird received 50 g of feed per day, then the quantity of feed increased 10 g every 2 days for 12 days. Following the termination of feed withdrawal, chickens were exposed to 14.5 L:9.5 D for 1 wk, and then light exposure time increased 30 min for every 2 wk up to 17 L:7 D.

Infection. An overnight heart infusion broth (Eiken, Tokyo, Japan) culture of a rifampicin-resistant mutant of SE HY-1, which was maintained as a frozen stock at −80 C, was used. All hens in groups 1 through 6 received an oral dose (by gavage) of 1 ml of this bacterial suspension (2.4×10^9 bacteria, as determined by dilution analysis) on day 4 of the feed withdrawal described above.

Egg production. Egg lay for each hen was recorded daily for 13 wk, and the rate (%) of egg production per week was calculated as number of eggs laid for 1 wk divided by 7 and multiplied by 100, and the means were calculated.

Bacteriology. Samples of cecal dropping (ca. 0.5 g) were taken at appropriate intervals from 1 day postinoculation (dpi) to 56 dpi from all hens. The

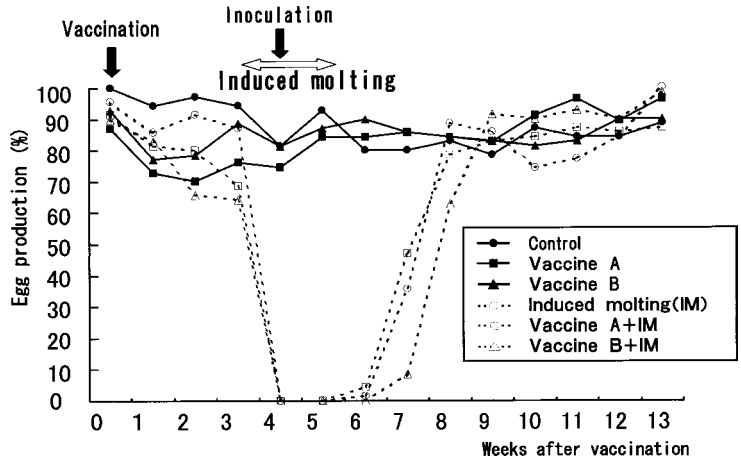

Fig. 1. Egg production in vaccinated and molted hens.

samples were diluted 1:10 (w:v) in Hajna tetrathionate (HTT) broth (Eiken) and homogenized. The broth was serially diluted (10-fold) in phosphate-buffered saline, and 0.025 ml of each dilution was spread onto desoxycholate–hydrogen sulfide–lactose (DHL) agar (Eiken) plates containing 100 μg/ml rifampicin. The sensitivity of detection was 400 cells/g of cecal dropping. Incubation was conducted at 37 C for 24 hr, and bacterial counts were made. For the direct enrichment culture, the broth was incubated at 41.5 C for 24 hr. The broth was then left at 25 C for 5–7 days for delayed secondary enrichment (DSE) (22). One milliliter of the broth was added to a fresh tube of HTT broth and incubated at 37 C overnight. One loopful of each broth was then streaked onto a DHL agar plate containing 100 μg/ml rifampicin and incubated at 37 C. The identity of prospective SE colonies was confirmed biochemically and serologically.

For the statistical analysis described below, it was assumed that 100 cells/g were contained in the cecal droppings when enrichment cultures were positive and that 10 cells/g were contained in the cecal dropping when DSE was positive.

Statistical analysis. The numbers of SE per milligram of fecal droppings were transformed to $\log_{10}$ and then means were calculated. Significant differences were calculated using the Student *t*-test.

RESULTS

Effect of vaccination on egg production. Egg production for all groups of hens throughout the experiment is shown in Fig. 1. Egg production decreased in the vaccinated hens 1–2 wk after bacterin administration. After feed withdrawal, egg production in hens of the molted groups (groups 4, 5, and 6) decreased rapidly and then ceased. Following resumption of feed, egg production gradually returned and attained normal levels of production 5 or 6 wk postinduction of molt (8 or 9 wk postvaccination). After that, egg production in all groups of hens remained at greater than 80% to the end of the experiment.

The total number of eggs laid in each group for the first 25 days and for 3 mo is shown in Fig. 2. For 25 days after vaccination, number of eggs laid in the vaccinated groups decreased ca. 15% compared with similar measures in the control group. For 3 mo after vaccination, hens in the two vaccinated groups (groups 2 and 3) exhibited a 7.7% and 3.0% reduction in egg lay, respectively, compared with those in the control group (group 1). As expected, egg production in the three molted groups (groups 4, 5, and 6) was less than one third of that observed for the unmolted/unvaccinated hens (group 1), but there were no significant difference in production within these three groups.

Viable counts of SE in cecal droppings. In the comparison of SE shedding between the control group (group 1) and the vaccinated and unmolted groups (groups 2 and 3), hens in groups 2 and 3 shed less SE than did the hens in control group. However, there are no significant differences between those values through the experiment, except for the sample that was examined 6 days after the challenge in group 3 (data not shown).

Shedding of SE into cecal droppings from hens of the control group (group 1) and the molted group (group 4) is shown in Fig. 3. Hens in the control group shed 5–6 logs SE/g on days 1–3. Thereafter,

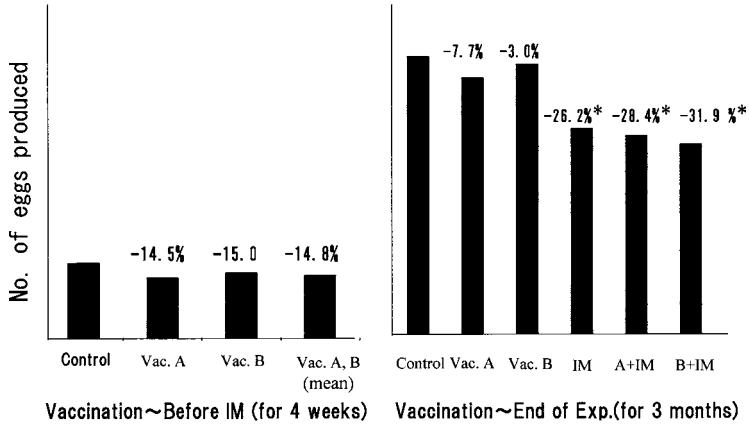

Fig. 2. Number of eggs in vaccinated and molted hens. The percent figures on top of the bars are no. of eggs reduced. * = Significantly different from control at $P < 0.05$.

the levels decreased rapidly to 2 logs of SE by day 9 and then decreased more gradually out to day 42. Conversely, hens in the molted group shed 7–8 logs SE/g on days 1–9 ($P < 0.01$ on days 3, 6, and 9), decreasing to 3 logs SE on day 14 ($P < 0.05$) and then down to control levels by day 21. No SE could be detected in either group of hens on day 49 or 56.

Cecal shedding of SE by hens of the molted group (group 4) and the vaccinated/molted groups (groups 5 and 6) is shown in Fig. 4. Hens in the vaccinated/molted groups shed about 2 logs less SE than did hens in the molted group on days 3–14 ($P < 0.05$ or 0.01). Thereafter, SE shedding was similar in all three groups.

DISCUSSION

In Japan, four products of SE-inactivated vaccines were commercially available: Layermune SE (imported from the United States), Inactivac SE4 (U.S.A.), Salenvac (U.K.), and Inactivated Vaccine (Japan). In the present study, Layermune and Inactivac SE4 were used.

The effects of injection of these vaccines on egg production in egg-laying hens were investigated. Because it is important to evaluate vaccination influences precisely, molting procedures were carried out according to those used by the supplier of the chickens (Aomori Poultry Co. Ltd.), a 300,000–laying hen operation. In the short term, the first 25 days after vaccination, eggs laid in the vaccinated groups decreased ca. 15%. However, after molting, egg production returned to the normal level. Over a longer period of lay, 3 mo, hens in the two vaccinated groups exhibited a 7.7% and 3.0% reduction in egg lay compared with those in the control group. Further, comparing egg production by the unvaccinated/molted group and the two vaccinated/molted groups showed no significant differences at the $P < 0.05$ level, a more acceptable decrease

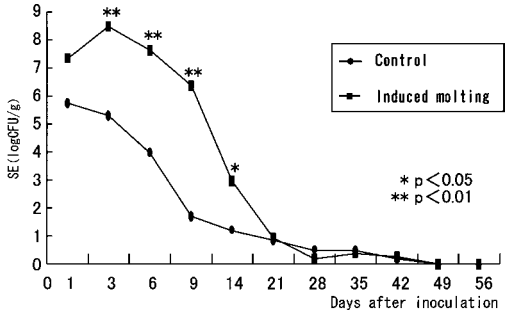

Fig. 3. Viable counts of SE in cecal droppings of control and molted hens.

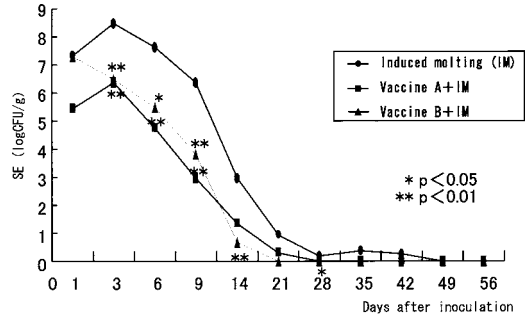

Fig. 4. Viable counts of SE in cecal droppings of vaccinated and molted hens.

in production. However, because of the group size of 10 birds in the present study, it might be necessary to carry out studies in larger group sizes in order to confirm whether or not vaccination really reduces egg lay significantly in the field. It is possible that the formulation of the vaccine and its adjuvant properties may potentially be stressful to hens at this age, thereby resulting in a reduction of egg production.

The effect of SE bacterins on reduction of viable SE counts in cecal droppings (15,23) and fecal samples (1,13) was investigated previously, and it was shown that chickens receiving these vaccines exhibited partial to marked reduction in SE shedding. In the present study, the vaccination did not always lead to significant reductions in shedding of SE by challenged hens, when comparing the control group and the vaccinated/unmolted groups. Therefore, the efficacy afforded by vaccination was only partial in unmolted hens.

On the other hand, hens in the molted groups shed significantly higher amounts of SE than did the hens of the control group, as shown in Fig. 3. This high level of SE shedding in molted groups might be due to the stress induced by molting, resulting in the alteration of T-lymphocyte subpopulations previously reported (2,3). However, the high levels of SE shedding observed in molted hens was improved when these hens had been vaccinated prior to molting. The mechanisms of the reduced SE shedding during molt in the vaccinated hens remain unclear, but the current results do indicate that vaccination with an SE bacterin prior to molt might be effective in preventing the exacerbation of SE problems within flocks in which the possibility of SE infection might be an issue.

A number of strategies have been described that might be implemented to ameliorate the SE problems in molted hens: combined use of an antibiotic and probiotic (19), feeding hens a low-energy, low-calcium diet in metered amounts (6,17), *ad libitum* wheat middings (18), or vaccination with a live attenuated *S. enterica* serovar *typhimurium* (7). It remains to be determined which methods, either singly or in combination, are most efficacious in reducing SE problems during molt under commercial situations, including vaccination of SE bacterin prior to molting.

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